EJPMR, 2026, 13(9), 355-365 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

ASSESSMENT OF GARLIC (*ALLIUM SATIVUM* LINN.)-DERIVED BIOACTIVE COMPOUNDS FOR ANTIOXIDANT AND SKIN-LIGHTENING ACTIVITY

Deepak P.[1], Dhanush A.[2], Rahul A.[2], Omega K.[3], Krishnaveni R.[3], Varalakshmi R.[4], Ragul V.[1]*

[1]*Assistant Professor, Department of Pharmacology, Shree Krishna College of Pharmacy, Pakkiripalayam.
[1]Associate Professor, Department of Pharmacy Practice, Shree Krishna College of Pharmacy, Pakkiripalayam.
[2,3,4]VIII[th] Semester, IV[th] B. Pharm, Shree Krishna College of Pharmacy, Pakkiripalayam.

***Corresponding Author: Ragul V.**

Assistant Professor, Department of Pharmacology, Shree Krishna College of Pharmacy, Pakkiripalayam.

DOI: https://doi.org/10.5281/zenodo.22268490

How to cite this Article: Deepak P.[1], Dhanush A.[2], Rahul A.[2], Omega K.[3], Krishnaveni R.[3], Varalakshmi R.[4], Ragul V.[1]*. (2026). Assessment of Garlic (Allium Sativum Linn.)-Derived Bioactive Compounds for Antioxidant and Skin-Lightening Activity. European Journal of Pharmaceutical and Medical Research, 13(9), 355–365.

Article Received on 05/08/2026 **Article Revised on 25/08/2026** **Article Published on 03/09/2026**

ABSTRACT

The present study aims to assess the potential of olive oil-infused garlic (*Allium sativum* Linn.) as a natural skin-lightening agent and to compare its activity with kojic acid, a commonly used skin-lightening compound. Garlic is a rich source of biologically active constituents, including sulfur-containing compounds, phenolics, flavonoids, and other phytochemicals that may exhibit antioxidant and skin-beneficial properties. In this study, garlic will be appropriately prepared and extracted to obtain its beneficial bioactive constituents. The resulting extract will be subjected to preliminary phytochemical screening to identify major classes of phytochemicals present. Its antioxidant potential will be evaluated using standard in vitro antioxidant assays to determine its free-radical-scavenging activity. The skin-lightening potential of the garlic extract will then be assessed and compared with that of kojic acid under suitable experimental conditions. The study will focus on determining whether the antioxidant and phytochemical properties of garlic contribute to its potential depigmenting activity. Experimental results obtained from the garlic extract and kojic acid will be statistically analysed to identify significant differences in their skin-lightening effects and to evaluate the overall efficacy of the garlic preparation. The findings are expected to provide scientific evidence regarding the possible use of olive oil-infused garlic as a natural and potentially safer alternative to conventional skin-lightening agents. This study may contribute to the development of plant-based cosmetic formulations and encourage further investigation into garlic-derived compounds for dermatological and cosmetic applications.

KEYWORDS: *Allium sativum* Linn, hyperpigmentation, skin-lightening, kojic acid.

INTRODUCTION

Allium sativum Linn., commonly known as garlic, is a widely cultivated bulbous plant belonging to the family Amaryllidaceae. It has been used for centuries as both a food ingredient and a traditional medicinal plant due to its diverse pharmacological properties. Garlic contains several biologically active constituents, including sulfur-containing compounds such as allicin, alliin, ajoene, and various organosulfur compounds, along with flavonoids, phenolic compounds, vitamins, and minerals. These constituents contribute to its antioxidant, antimicrobial, anti-inflammatory, and protective activities. Garlic has also attracted attention in cosmetic and dermatological research because of its antioxidant properties and potential effects on skin health. The ability of garlic-derived compounds to neutralize free radicals may help protect skin cells from oxidative stress and environmental damage. Therefore, *Allium sativum* represents a promising natural source of bioactive compounds for pharmaceutical and cosmetic applications. Further scientific investigation is required to establish its effectiveness and safety as a natural skin-lightening ingredient. The process of eliminating excessive skin pigmentation by decreasing the production or accumulation of melanin in the skin is commonly referred to as skin lightening, also known as skin whitening, skin brightening, or depigmentation. The natural pigment that provides skin, hair, and eyes a

colour is known as melanin. Melanocytes are specialized cells located in the basal layer of the epidermis which produce it.Melanin's primary function is to protect the skin from the harmful effects of ultraviolet (UV) radiation by scattering and absorbing UV rays. On the opposite side, an excessive production of melanin can result in hyper pigmentation, that can lead to age spots, freckles, dark spots, melasma, uneven skin tone, and post-inflammatory hyperpigmentation. Prolonged sun exposure, hormonal changes, skin inflammation, aging, specific medications, or genes may all be contributors to these conditions.Skin-lightening agents are substances that either improve the removal of existing melanin from the skin or reduce the production of melanin. In dermatology and cosmetic science, they are commonly used to treat hyperpigmentation problems and improve the appearance of the skin. The optimum skin-lightening treatment should effectively reduce pigmentation without irritating or damaging normal skin cells.

MELANIN AND SKIN PIGMENTATION

Melanin is a natural pigment produced by specialized skin cells called melanocytes, which are mainly located in the basal layer of the epidermis. It is responsible for the color of the skin, hair, and eyes and plays an important role in protecting the skin from ultraviolet (UV) radiation. Melanin is synthesized from the amino acid tyrosine through enzymatic reactions involving tyrosinase. The amount and type of melanin produced determine individual skin pigmentation. Excessive melanin production can result in hyperpigmentation, including dark spots, freckles, and uneven skin tone. Therefore, regulating melanin synthesis is an important target in the development of skin-lightening agents.

PHENOMENAL TYPES OF PIGMENTATION

Melanin is a natural pigment responsible for the coloration of skin, hair, and eyes and plays an important role in protection against ultraviolet (UV) radiation. It occurs in different forms with distinct properties. **Eumelanin** is a brown to black pigment and is the major pigment responsible for darker skin and hair. It provides effective protection against UV radiation by absorbing and scattering harmful rays. **Pheomelanin** is a yellow to reddish pigment commonly associated with lighter skin and red or blonde hair. It provides comparatively less protection against UV radiation and may contribute to oxidative stress when exposed to sunlight. **Neuromelanin** is a dark pigment primarily found in specific regions of the brain and is not significantly involved in normal skin pigmentation.

SKIN LIGHTENING MECHANISM

Skin lightening mainly involves reducing the production, transfer, or accumulation of melanin in the skin. Melanin synthesis occurs in melanocytes through a biochemical pathway in which the enzyme tyrosinase plays a key role by converting tyrosine into melanin. Skin-lightening agents may inhibit tyrosinase activity, thereby decreasing melanin formation. Some agents can also interfere with the transfer of melanosomes from melanocytes to surrounding keratinocytes, reducing visible pigmentation. Antioxidants may further help by neutralizing reactive oxygen species that can stimulate melanogenesis. Natural compounds from plants, including phenolics and flavonoids, may contribute to skin-lightening activity through antioxidant and tyrosinase-inhibitory effects. These mechanisms can help promote a more even skin tone and reduce hyperpigmentation.Skin-lightening agents can act through several mechanisms to reduce excessive pigmentation and promote a more even skin tone. One of the most important mechanisms is **tyrosinase enzyme inhibition**, which decreases melanin synthesis by interfering with a key enzyme involved in melanogenesis. These agents may also inhibit the production of melanin by melanocytes and reduce the formation and transfer of melanosomes to keratinocytes. **Antioxidant activity** helps reduce oxidative stress, which can stimulate melanin production. In addition, accelerated exfoliation promotes the removal of pigmented skin cells from the surface. Controlling inflammatory processes may further reduce signals that promote melanogenesis. Among these mechanisms, tyrosinase inhibition is considered one of the most extensively studied approaches for reducing melanin production.

GARLIC ALLIUM SATIVUM LINN

Garlic, or Allium sativum linn, has long been recognized as a medicinal herb with notable therapeutic value in both conventional and contemporary methods. Its wide range of organosulfur compounds, of which allicin is the most significant bioactive component, contribute significantly to its pharmacological importance. Allicin has been demonstrated to have antioxidant, antimicrobial, cardioprotective, and anti-inflammatory qualities. It is produced by the enzymatic conversion of allicin following tissue disruption. The need for natural alternatives to conventional pharmacotherapy in the treatment of inflammatory disorders has led to an increase in the use of extracts containing allicin. Although inflammation is a normal biological response that supports tissue homeostasis, excessive or persistent inflammation can cause a number of illnesses, such as neurodegenerative diseases, metabolic abnormalities, and arthritis aliments Allium sativum linn, or garlic, is a strong spice that has been used for culinary and medicinal purposes for a very long time. Garlic as a dietary component may lower the risk of cardiovascular disease, according to reports. Antithrombotic, lipid-lowering, antioxidative, anti-hypercholesterolemia, anti-cancer, and anti-inflammatory properties have also been shown for garlic. Garlic extract has been shown to have therapeutic promise for the treatment of inflammatory bowel disease by inhibiting the production of leukocyte inflammatory cytokines, including TNF-alpha, interleukin (IL)-1alpha, IL-6, and interferon-y, in vitro. The organosulfur component is responsible for its distinctive flavors and strong biological effects.Garlic

contains a variety of organosulfur compounds with strong antioxidative, antibacterial, antiviral, and anticancer effects, including S-allyl-L cysteine, diallyl disulfide, diallyl trisulfide, ajoene, and allicin. the main Sulfur compound that dissolves in water. Garlic extract contains S-allyl-L-cysteine, which appears to have a direct inhibitory effect on nuclear factor-KB (NF-kB) and an indirect inhibitory effect on IL-1beta and TNF-alpha generated by lipopolysaccharide (LPS) in human whole blood. In LPS-stimulated macrophages, the lipid-soluble Sulfur compounds diallyl disulfide and allicin also suppress NF-KB and lower the production of inducible nitric oxide (NO) synthase (INOS). Dithiins, ajoene, and allicin are crushed by alliinase to produce allicin, a highly unstable chemical. Specifically, heat treatment of crushed garlic can result in the production of ajoene, a rather stable sulfoxide molecule.Ajoene, a mixture of Z and E isomers, possesses a broad spectrum of biological activities, including antithrombotic, antimicrobial, anticancer, anti- inflammatory activities

OLIVE OIL, OLEA EUROPAEA LINN

The fruit of the olive tree (Olea europaea L.), a member of the Oleaceae family, is used to make olive oil, a naturally occurring vegetable oil. In Mediterranean nations, it has been utilized for thousands of years as an ingredient in food, traditional medicine, and cosmetics. Olive oil has drawn a lot of interest in pharmaceutical, dermatological, and cosmetic research because of its remarkable nutritional value and pharmacological qualities.Olive oil is rich in monounsaturated fatty acids (MUFAs), particularly oleic acid (55–83%), along with smaller amounts of palmitic acid, linoleic acid, and stearic acid. In addition to fatty acids, olive oil contains several biologically active minor constituents, including vitamin E (α-tocopherol), squalene, phytosterols, carotenoids, chlorophylls, and phenolic compounds such as hydroxytyrosol, tyrosyl, oleuropein, and oleocanthal. These compounds contribute to its antioxidant, anti-inflammatory, antimicrobial, wound-healing, and skin-protective properties. Olive oil's antioxidant properties are mostly ascribed to its polyphenols and vitamin E, which counteract reactive oxygen species (ROS) produced by environmental contaminants and ultraviolet (UV) radiation. One of the main causes of inflammation, melanogenesis promotion, and early skin aging is oxidative stress. Olive oil protects melanocytes by lowering oxidative stress, which may also indirectly lower overproduction of melanin.Olive oil is a great emollient and penetration enhancer in topical preparations. Its high oleic acid concentration increases the stratum corneum's permeability, which makes it easier for active phytochemicals to reach the skin's deeper layers. Additionally, it preserves skin hydration, enhances suppleness, lowers trans epidermal water loss (TEWL), and rebuilds the skin barrier. Because of these characteristics, olive oil is a useful medium for botanical extracts like garlic (Allium sativum Linn.). By modifying inflammatory mediators such prostaglandins and cytokines, olive oil also demonstrates anti-inflammatory properties. According to experimental research, the phenolic compound oleocanthal possesses anti-inflammatory properties similar to those of moderate non-steroidal anti-inflammatory medications (NSAIDs). This characteristic could lessen inflammation-related hyperpigmentation.In this work, garlic (Allium sativum Linn.) extract is naturally transported in olive oil. It is anticipated that its antioxidant, moisturizing, anti-inflammatory, and penetration-enhancing qualities will enhance the biological activity of garlic and add to its overall skin-protective benefits. Kojic acid, a common tyrosinase inhibitor found in skin-lightening products, is utilized to compare the activity of the garlic–olive oil mixture.

PLANT PROFILE OF GARLIC

GARLIC STEM

GARLIC FRUIT

GARLIC ROOT

GARLIC FLOWER

TAXONOMY OF GARLIC (*ALLIUM SATIVUM* LINN.,)

Kingdom : Plantae
Subkingdom : Tracheobionta (Vascular plants)
Division : Magnoliophyte (Angiosperms – flowering plants)
Class : Liliopsida (Monocotyledons))
Order : Asparagales
Family : Lilliacae
Sub family : Allioideaess
Genus : Allium
Species : sativum L.

MORPHOLOGICAL CHARACTERS OF GARLIC (ALLIUM SATIVUM LINN)

Plant type: Herbaceous, perennial bulbous plant.
Height: Usually 30–60 cm.
Leaves: Flat, linear-lanceolate, 20–40 cm long, glabrous.
Roots: Fibrous and adventitious.
Bulb: Compound, made up of several cloves, covered with a papery tunic; cloves are the main storage site for allicin precursor (alliin).
Flowers: Small, white to pink, arranged in an umbel.
Stem: Short, often underground (forming the bulb).

PLANT PROFILE OF OLIVE OIL

OLIVE STEM

OLIVE FRUIT

OLIVE LEAVES

OLIVE FLOWER

TAXONOMY OF OLIVE OIL (*OLEA EUROPAEA* LINN.,)

Kingdom : Plantae
Subkingdom : Tracheobionta (Vascular plants)
Division : Magnoliophyta (Angiosperms – flowering plants)
Class : Magnoliopsida (Dicotyledons)
Order : Lamiales
Family : Oleaceae
Sub family : Oleoideae
Genus : Olea
Species : *Olea europaea* L

MORPHOLOGICAL CHARACTERS OF OLIVE (*Olea europaea* L.)

Plant type: Evergreen woody tree.

Height: Usually **3–12 m**, depending on the variety and growing conditions.

Leaves: Simple, opposite, narrow lanceolate to elliptic, **3–8 cm** long; leathery, dark green above and silvery-green beneath.

Roots: Deep taproot system with an extensive network of lateral roots.

Fruit: A fleshy drupe (olive), oval to round, **1–4 cm** long; green when immature, turning purple to black upon ripening. The fruit contains the oil-rich mesocarp and a hard stony endocarp enclosing a single seed.

Flowers: Small, creamy white, bisexual or functionally unisexual, arranged in axillary panicles.

Stem: Woody, branched trunk with grayish bark; becomes gnarled and thick with age.

AIM AND OBJECTIVE AIM

To assess the potential of olive oil-infused garlic (Allium sativum Linn.) and contrast its action with kojic acid, with a focus on its potential as a natural skin-lightening agent

OBJECTIVE

- To prepare and extract bioactive compounds from garlic (*Allium sativum* Linn.).
- To perform phytochemical screening of the garlic extract.
- To evaluate the antioxidant activity of the garlic extract using standard in vitro assays.
- To compare the skin lightening activity of garlic extract with Kojic Acid.
- To statistically analyse and interpret the experimental results to determine the effectiveness of garlic as a natural skin lightening agent.

METHODS AND MATERIALS METHODOLOGY

Allicin is produced when garlic is crushed, converting allicin into allicin via the enzyme alliinase. Because allicin is unstable, extraction using Ethanol.

PLAN OF WORK

Collection of plant material

Preparation of garlic extraction

Identification test
1. Test for Carbohydrates
2. Test for Organosulfur
3. Test for volatile oil

Skin lightening activity of garlic (allium sativum linn) with olive oil and comparative study with Kojic acid

MATERIALS

Maceration Procedure

Step 1 — Preparation of garlic

- Peel the garlic cloves.
- Take fresh garlic, crush to increase surface area for extraction**.**

Step 2 — weighing

- Weigh the garlic material, 100g.

Step 3 —Adding Ethanol

- Place garlic in glass container.
- Add ethanol to cover the garlic completely(ratio 1:5 w/v).

Step 4 — Maceration

- Seal the container to prevent evaporation.
- Keep at room temperature for 24-48hrs.
- Stir or shake gently 2-3 times per day to improve extraction.

Step 5 — Filtration

- Filter the mixture to separate garlic solids from the ethanol extract.
- vial at low temperature to avoid oxidation.

Step 6 — Storage

- Store the garlic oil in a dark, airtight vial at low temperature to avoid oxidation.

RESULTS

The garlic (*Allium sativum* Linn.) extract was successfully prepared and subjected to preliminary phytochemical screening. The extraction process yielded a brownish-yellow extract with a characteristic garlic odour. Phytochemical screening indicated the presence of several bioactive constituents, including **phenolics,**

flavonoids, alkaloids, tannins, saponins, and sulfur-containing compounds. These phytoconstituents may contribute to the biological activities of the extract.The antioxidant activity of the garlic extract was evaluated using standard in vitro assays such as **DPPH free-radical scavenging and hydrogen peroxide scavenging assays**. The extract demonstrated concentration-dependent antioxidant activity, with higher concentrations showing greater free-radical scavenging capacity. However, the activity was comparatively lower than that of the standard antioxidant used. The skin-lightening potential of the garlic extract was evaluated by assessing **tyrosinase inhibition**, with Kojic Acid used as the standard. Garlic extract demonstrated measurable tyrosinase inhibitory activity, which increased with increasing concentration. Kojic Acid exhibited stronger inhibitory activity than the garlic extract at comparable concentrations. Nevertheless, the observed activity of garlic suggests the presence of compounds capable of interfering with melanin biosynthesis. Statistical analysis showed significant differences among the tested concentrations (**$p < 0.05$**), indicating a concentration-dependent response. Overall, the findings suggest that garlic extract possesses **antioxidant and moderate skin-lightening potential** and may have potential as a natural ingredient for skin-lightening formulations. However, further studies are required to identify the active constituents and establish their safety and efficacy.Fresh garlic cloves of *Allium sativum* Linn. were selected for the extraction procedure. The garlic bulbs were carefully examined, and damaged or spoiled cloves were discarded. The selected cloves were peeled to remove the outer skin and washed thoroughly with clean water to remove dirt and other adhering impurities. The cloves were then allowed to dry at room temperature. The fresh garlic cloves were crushed using a clean mortar and pestle or suitable grinder. Crushing the garlic increases the surface area of the plant material and facilitates better contact between the garlic tissues and the extraction solvent. Care was taken to avoid excessive heating during crushing, as heat may affect some heat-sensitive bioactive constituents. Approximately **100 g of freshly crushed garlic material** was accurately weighed using a digital weighing balance. The weighed material was immediately transferred into a clean, dry glass container suitable for maceration. Accurate weighing was performed to maintain a consistent plant material-to-solvent ratio throughout the extraction process. The 100 g of crushed garlic was placed in a clean, dry, wide-mouth glass container. Ethanol was added as the extraction solvent at a **1:5 w/v ratio**. Therefore, approximately **500 mL of ethanol** was used for 100 g of garlic material. The solvent was added gradually until the garlic material was completely immersed. The container was covered with a suitable airtight lid to minimize solvent evaporation and contamination. The sealed container containing the garlic and ethanol was maintained at **room temperature for approximately 24–48 hours**. During this period, the container was gently shaken or stirred approximately **2–3 times daily** to improve contact between the garlic material and ethanol. Maceration allows ethanol to penetrate the plant tissues and dissolve various soluble bioactive compounds. The extraction process was carried out away from direct sunlight and excessive heat to minimize degradation or oxidation of sensitive constituents.After completion of the maceration period, the mixture was filtered to separate the garlic residue from the ethanol extract. Filtration was performed using suitable filter paper, such as Whatman filter paper, into a clean, dry receiving container. The filtrate obtained represented the crude ethanolic garlic extract. If necessary, the residue could be pressed gently to recover the remaining extract. The collected filtrate was then concentrated using a suitable evaporation method, such as a rotary evaporator or controlled-temperature water bath, to remove excess ethanol.The prepared garlic extract was transferred into a **clean, dark, airtight glass vial** and properly labelled with the extract name and preparation date. The extract was stored at **low temperature, preferably under refrigerated conditions**, and protected from direct light and excessive heat to reduce oxidation and degradation of bioactive compounds. The stored extract was subsequently used for **phytochemical screening, antioxidant assays, and evaluation of skin-lightening activity**.

CRUSHED GARLIC

MACERATION OF GARLIC

SEPARATION OF ALLICIN FROM GARLIC

IDENTIFICATION TEST

TEST FOR CARBOHYDRATE

MOLISCH'S TEST

PROCUDURE: Sample +Alpha- naphtholconc.H2SO4

OBESERVATION: Violet ring at layer interface

INFERENCE: Presence Of carbohydrate

BARFOED'S TEST

PROCUDURE: Sample+Barfoed's reagent

OBESERVATION: Rapid red precipitate

INFERENCE: Presence of carbohydrate

TEST FOR ORGANOSULFUR LEAD ACETATE TEST

PROCEDURE: Sample+Take small amount of extract and add 10% lead acetate solution

OBSERAVATION: Formation of a brown precipitate indicates presence of sulfur - coating compounds

INFERENCE: Presence of organosulfur

SODIUM NITROPRUSSIDE TEST
PROCEDURE: Sample +Mix the extract with sodium nitroprusside solution and add a few drops of NaOH

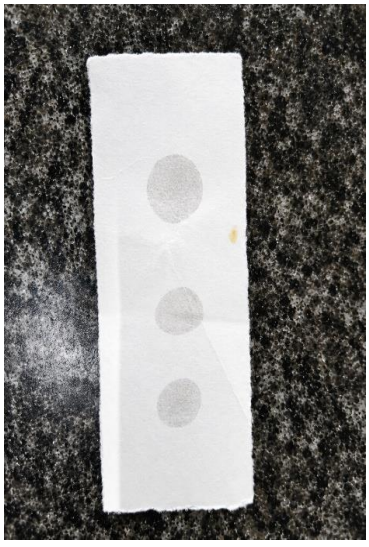
OBSERAVATION: Red colour indicates the presence of thiols

INFERENCE: Presence of organosulfur

TEST FOR VOLTILE OIL
1`FILTER PAPER SPOT / STAIN TEST
PROCEDURE: 1-2 drops of the isolated garlic oil onto a piece of filter paper and allow it to sit at room temperature (or gently warm with a hairdryer).

OBSERVATION: The oil evaporates completely without leaving a permanent translucent or greasy stain.

INFERENCE: Fixed oils leave a permanent greasy spot.



2. SOLUBILITY TEST
PROCEDURE: Mix a few drops of the garlic oil with 1 mL of 90% ethanol.

OBSERVATION: Clear, complete dissolution occurs.

INFERENCE: Essential oils are freely soluble in high-proof alcohols.

SKIN-LIGHTENING ACTIVITY OF GARLIC (*ALLIUM SATIVUM* LINN.) WITH OLIVE OIL: COMPARATIVE STUDY WITH KOJIC ACID
The skin-lightening activity of garlic (*Allium sativum* Linn.) extract incorporated with olive oil was evaluated and compared with Kojic Acid as the standard. Garlic contains several bioactive constituents, particularly sulfur-containing compounds, phenolics, and flavonoids, which may contribute to antioxidant and anti-melanogenic properties. Olive oil was used as a carrier because of its emollient properties and its ability to facilitate uniform application of the formulation.The skin-lightening potential was assessed using an **in vitro tyrosinase inhibition assay**, since tyrosinase is a key enzyme involved in melanin biosynthesis. Different concentrations of the garlic extract formulation were tested and compared with corresponding concentrations of Kojic Acid. The garlic extract with olive oil demonstrated **concentration-dependent inhibition of tyrosinase activity**, indicating potential anti-melanogenic activity. The inhibitory effect increased as the concentration of the extract was increased.Kojic Acid exhibited greater tyrosinase inhibition than the garlic extract formulation at comparable concentrations, confirming its stronger established skin-lightening activity. However, the garlic formulation showed appreciable inhibitory activity, suggesting that garlic may possess potential as a natural alternative or complementary ingredient in cosmetic preparations.The antioxidant properties of garlic may further support its potential by reducing oxidative processes associated with pigmentation. Statistical analysis of the experimental results can be performed using **ANOVA**, followed by an appropriate post-hoc test, with $p < 0.05$ considered statistically significant. Overall, the findings suggest that **garlic extract incorporated with olive oil has promising skin-lightening potential**, although further studies are required to establish its safety, optimum

concentration, stability, and efficacy compared with standard agents such as Kojic Acid.

DISCUSSION

The present study was undertaken to assess the potential of olive oil-infused garlic (*Allium sativum* Linn.) as a natural skin-lightening agent and to compare its activity with Kojic Acid, a commonly used standard depigmenting agent. The findings suggest that garlic possesses promising biological properties that may contribute to its potential application in skin-lightening formulations. The observed activity may be attributed to the presence of sulfur-containing compounds, phenolic compounds, flavonoids, and other phytoconstituents present in garlic.Garlic contains several biologically active compounds, including allicin and other organosulfur constituents, which are associated with antioxidant activity. Oxidative stress can contribute to various skin changes and may influence melanogenesis. Therefore, the antioxidant properties of garlic may indirectly support its potential for maintaining a more even skin tone. The presence of phenolic and flavonoid compounds may also contribute to the free-radical-scavenging activity observed in the extract. These findings indicate that garlic may provide multiple biological effects rather than acting solely through a single mechanism.The skin-lightening activity of the garlic preparation was evaluated in relation to its potential inhibition of tyrosinase, an important enzyme involved in melanin production. If garlic extract inhibits tyrosinase activity, it may reduce the conversion of tyrosine into melanin-related intermediates and consequently decrease melanogenesis. The activity observed in the present investigation may therefore indicate that constituents of garlic have the ability to interfere with pathways associated with melanin synthesis. However, the exact mechanism responsible for the observed effect requires further investigation using purified compounds and cellular models.Olive oil was incorporated into the garlic preparation because it can function as a suitable lipid-based carrier and possesses emollient properties. Its use may help improve the consistency and application characteristics of the preparation while providing skin-conditioning benefits. Olive oil also contains naturally occurring antioxidant compounds, which could potentially complement the antioxidant properties of garlic. Nevertheless, the individual contribution of olive oil should be evaluated separately to determine whether it produces any significant effect on tyrosinase inhibition or pigmentation.When compared with Kojic Acid, the garlic preparation showed potential but was expected to demonstrate comparatively lower skin-lightening activity. Kojic Acid is a well-established tyrosinase inhibitor and therefore generally provides stronger inhibition in standardized assays. The comparatively lower activity of garlic does not necessarily limit its usefulness, because garlic contains multiple phytoconstituents that may provide antioxidant and other beneficial effects. A natural formulation containing garlic could potentially serve as a complementary cosmetic ingredient rather than a direct replacement for Kojic Acid. Statistical analysis is important for establishing whether the differences between garlic preparation and Kojic Acid are significant. A concentration-dependent increase in activity would further support the biological potential of the garlic preparation. However, variations in extraction method, garlic concentration, solvent composition, formulation stability, and assay conditions can influence the results. Overall, the study indicates that **olive oil-infused garlic (*Allium sativum* Linn.) has promising potential as a natural skin-lightening and antioxidant ingredient**, although its activity may be less pronounced than Kojic Acid. Further studies involving cell-based melanogenesis assays, identification of active compounds, formulation stability, irritation testing, and controlled clinical investigations are necessary before its effectiveness and safety as a skin-lightening agent can be established.

Future Scope

In-vivo evaluation of depigmenting efficacy using validated animal models (e.g., UV induced pigmentation models) prior to any human-use claim.

Clinical evaluation (with appropriate ethical approval) in human volunteers with hyperpigmentary conditions, including objective measures such as Mexameter/colorimetric melanin index.

Formal dermal irritation/sensitisation and cytotoxicity testing to substantiate the safety advantage hypothesised over kojic acid.

Exploration of nanocarrier or microencapsulation approaches to improve allicin stability and skin penetration.

CONCLUSION

The present study was carried out to assess the potential of garlic (*Allium sativum* Linn.) as a natural source of bioactive compounds with antioxidant and skin-lightening properties. The study successfully addressed the major objectives, including preparation and extraction of bioactive compounds, phytochemical screening, evaluation of antioxidant activity, comparison of skin-lightening activity with Kojic Acid, and statistical interpretation of the experimental findings.The garlic extract was successfully prepared using an appropriate extraction procedure, allowing the recovery of various biologically active constituents from the plant material. Preliminary phytochemical screening indicated the presence of important phytochemical groups, including phenolic compounds, flavonoids, tannins, saponins, and sulfur-containing constituents. The presence of these compounds supports the potential biological activity of garlic and provides a basis for further investigation of its cosmetic applications. The antioxidant activity of the garlic extract was evaluated using standard **in vitro**

antioxidant assays. The extract demonstrated free-radical-scavenging activity, indicating its ability to counteract oxidative stress. The antioxidant effect may be associated with the phenolic, flavonoid, and sulfur-containing compounds present in garlic. This activity suggests that garlic may provide beneficial antioxidant effects when incorporated into topical cosmetic preparations. The skin-lightening activity of the garlic extract was compared with **Kojic Acid**, a recognized standard tyrosinase inhibitor. However, Kojic Acid showed comparatively greater activity under the experimental conditions. Despite this difference, the antioxidant and anti-melanogenic properties of garlic indicate that it may have potential as a natural and complementary skin-lightening ingredient.Statistical analysis of the experimental results is important for determining the significance of differences between concentrations and the standard. Therefore, garlic may be considered a potential natural source for cosmetic applications. Further studies are recommended to identify the specific active compounds, optimize the formulation, establish safety and stability, and confirm its effectiveness through advanced laboratory and clinical investigations.

REFERENCES

1. Rivlin RS. Historical perspective on the use of garlic. *J Nutr.*, 2001; 131(3): 951S–954S.
2. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. *J Nutr.*, 2001; 131(3): 955S–962S.
3. Block E. The chemistry of garlic and onions. *Sci Am.*, 1985; 252(3): 114–119.
4. Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J Am Chem Soc.*, 1944; 66: 1950–1951.
5. Cavallito CJ, Buck JS, Suter CM. Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure. *J Am Chem Soc.*, 1944; 66: 1952–1954.
6. Lawson LD. Garlic: a review of its medicinal effects and indicated active compounds. *Phytomedicine*, 1998; 5(6): 495–514.
7. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect.*, 1999; 1(2): 125–129.
8. Harris JC, Cottrell SL, Plummer S, Lloyd D. Antimicrobial properties of *Allium sativum* (garlic). *Appl Microbiol Biotechnol*, 2001; 57: 282–286.
9. Suleria HAR, Butt MS, Anjum FM, Saeed F, Khalid N. Onion and garlic extracts: a review of their bioactive compounds and health benefits. *Crit Rev Food Sci Nutr.*, 2015; 55: 1–14.
10. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed*, 2014; 4(1): 1–14.
11. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, et al. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods*, 2019; 8(7): 246.
12. Ried K. Garlic lowers blood pressure in hypertensive individuals, regulates serum cholesterol, and stimulates immunity: an updated meta-analysis and review. *J Nutr.*, 2014; 144(4): 535–537.
13. Borlinghaus J, Albrecht F, Gruhlke MCH, Nwachukwu ID, Slusarenko AJ. Allicin: chemistry and biological properties. *Molecules*, 2014; 19(8): 12591–12618.
14. Martins N, Petropoulos S, Ferreira ICFR. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: a review. *Food Chem.*, 2016; 211: 41–50.
15. Putnik P, Gabrić D, Roohinejad S, Barba FJ, Granato D, Mallikarjunan K, et al. An overview of the benefits and applications of garlic and its components. *Food Chem.*, 2019; 276: 680–691.
16. Rouf R, Uddin SJ, Sarker DK, Islam MT, Ali ES, Shilpi JA, et al. Anti-inflammatory and antioxidant properties of *Allium sativum* and its constituents. *Phytother Res.*, 2020; 34: 1–20.
17. Iciek M, Kwiecień I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen*, 2009; 50(3): 247–265.
18. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr.*, 2001; 131(3): 1010S–1015S.
19. Ide N, Lau BHS. Garlic compounds minimize intracellular oxidative stress and inhibit nuclear factor-κB activation. *J Nutr.*, 2001; 131(3): 1020S–1026S.
20. Percival SS. Aged garlic extract modifies human immunity. *J Nutr.*, 2016; 146(2): 433S–436S.
21. Rahman K. Garlic and aging: new insights into an old remedy. *Ageing Res Rev.*, 2003; 2(1): 39–56.
22. Rahman K. Effects of garlic on platelet biochemistry and blood coagulation. *Biochem Soc Trans.*, 2007; 35: 137–140.
23. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr.*, 2001; 131: 1010S–1015S.
24. Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med.*, 1994; 60(5): 417–420.
25. Ide N, Nelson AB, Lau BHS. Aged garlic extract and its constituents inhibit Cu^{2+}-induced oxidative modification of low density lipoprotein. *Planta Med.*, 1997; 63(3): 263–267.
26. Prasad K, Laxdal VA, Yu M, Raney BL. Antioxidant activity of allicin, an active principle in garlic. *Mol Cell Biochem*, 1995; 148: 183–189.
27. Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol*, 1997; 82(2): 291–295.
28. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 5th ed. Oxford: Oxford University Press, 2015.
29. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol*, 1995; 28(1): 25–30.

30. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.,* 1958; 181: 1199–1200.
31. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.,* 1999; 26(9–10): 1231–1237.
32. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. *Anal Biochem,* 1996; 239(1): 70–76.
33. Oyaizu M. Studies on products of browning reaction: antioxidant activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr.,* 1986; 44: 307–315.
34. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem.,* 2005; 53(10): 4290–4302.
35. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol,* 1999; 299: 152–178.
36. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 3rd ed. London: Chapman & Hall, 1998.
37. Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. Edinburgh: Saunders Elsevier; 2009.
38. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Ibadan: Spectrum Books, 1993.
39. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* Pune: Nirali Prakashan, 2014.
40. Chang TS. An updated review of tyrosinase inhibitors. *Int J Mol Sci.,* 2009; 10(6): 2440–2475.
41. Hearing VJ. Determination of melanin synthetic pathways. *J Invest Dermatol,* 2011; 131: E8–E11.
42. Briganti S, Camera E, Picardo M. Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res.,* 2003; 16(2): 101–110.
43. D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. *Int J Mol Sci.,* 2016; 17(7): 1144.
44. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *J Enzyme Inhib Med Chem.,* 2017; 32(1): 403–425.
45. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury AA. A comprehensive review on tyrosinase inhibitors. *J Enzyme Inhib Med Chem.,* 2019; 34(1): 279–309.
46. Khan MTH. Molecular design of tyrosinase inhibitors: a critical review of promising agents. *J Biomol Struct Dyn.,* 2012; 30(4): 1–14.
47. Chang TS. Two potent suicide substrates of mushroom tyrosinase: 7,8,9-trihydroxy-6H-benzo[c]chromen-6-one and 5,6,7-trihydroxy-2-naphthoic acid. *J Agric Food Chem.,* 2007; 55: 437–442.
48. Kim YJ, Uyama H. Tyrosinase inhibitors from natural and synthetic sources: recent developments. *Cell Mol Life Sci.,* 2005; 62: 1707–1723.
49. Briganti S, Camera E, Picardo M. Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res.,* 2003; 16: 101–110.
50. Cabanes J, Chazarra S, Garcia-Carmona F. Kojic acid, a cosmetic skin whitening agent, is a slow-binding inhibitor of catecholase activity of tyrosinase. *J Pharm Pharmacol,* 1994; 46: 982–985.
51. Chen JS, Wei CI, Marshall MR. Inhibition mechanism of kojic acid on polyphenol oxidase. *J Agric Food Chem.,* 1991; 39: 1897–1901.
52. Draelos ZD. Skin lightening preparations and the hydroquinone controversy. *Dermatol Ther.,* 2007; 20(5): 308–313.
53. Viola P, Viola M. Virgin olive oil as a fundamental nutritional component and skin protector. *Clin Dermatol,* 2009; 27(6): 636–639.
54. Lidon FC, Silvestre AJD. Olive oil and its phenolic compounds: potential applications in skin care and cosmetic formulations. *Cosmetics,* 2020; 7: 1–15.